Efficacy of recombinant adenoviral human p53 gene in the treatment of lung cancer-mediated pleural effusion

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Abstract. Pleural effusion induced by lung cancer exerts a negative impact on quality of life and prognosis. The aim of the present study was to evaluate the value of the recombinant adenoviral human p53 gene (rAd-p53) in the local treatment of lung cancer and its synergistic effect with chemotherapy. The present study retrospectively recruited 210 patients with lung cancer-mediated pleural effusion who had adopted a treatment strategy of platinum chemotherapy. Pleurodesis was performed via the injection of cisplatin or rAd-p53. Long-term follow-up was conducted to investigate the therapeutic effects of cisplatin and rAd-p53 administration on pleural effusion and other relevant clinical indicators. The short-term effect of pleurodesis was as follows: The efficacy rate of rAd-p53 therapy was significantly higher compared with cisplatin therapy (71.26 vs. 54.47%), and the efficacy of treatment with $\geq 2 \times 10^{12}$ viral particles of rAd-p53 for pleurodesis was significantly greater than treatment with 40 mg cisplatin ($P < 0.05$). Furthermore, efficacy analysis performed 6 and 12 months after pleurodesis indicated that the efficacy rate of rAd-p53 was significantly greater than that of cisplatin ($P < 0.05$). A comparison of median progression-free survival (PFS) time identified a significant difference ($P < 0.05$) between rAd-p53 and cisplatin therapy (3.3 vs. 2.7 months); however, a comparison of median overall survival time identified no significant difference ($P > 0.05$) between rAd-p53 and cisplatin therapy (9.6 vs. 8.7 months). In addition, Cox regression analysis indicated that PFS was not affected by clinical indicators such as age, gender, prognostic staging and smoking status; however, PFS was affected by pathological subtype (adenocarcinoma or squamous carcinoma) in the rAd-p53 group. rAd-p53 administration for pleurodesis exerts long-term therapeutic effects on the local treatment of lung cancer. Thus, a combination of rAd-p53 and chemotherapy may exert a synergistic effect and reverse multidrug resistance.

Introduction

Lung cancer is the malignant tumor with the highest morbidity and mortality rates. The quality of life and survival time of patients with lung cancer are affected by various factors, including pleural effusion. The detection of malignant cells in plural effusion and/or parietal pleura indicates the presence of disseminated or advanced disease, as well as a reduced life expectancy in patients with cancer. Median survival time following a diagnosis of cancer-mediated pleural effusion is 3-12 months. Lung cancer-mediated pleural effusion survival time is even shorter (1-3), as patients often exhibit symptoms, such as chest distress, shortness of breath and inability to lie prostrate. If such symptoms are not promptly alleviated, they typically develop into respiratory dysfunction, hypoproteinemia and anemia, and even cause mortality. Thus, the treatment of pleural effusion is important for improving the quality of life and survival time of lung cancer patients.

Current lung cancer-mediated pleural effusion therapies include standard chemotherapy and local treatment. The underlying principles for pleural effusion therapies are described in the guidelines of the British Thoracic Society (1): Pleural effusions treated by aspiration alone are associated with a high rate of recurrence of effusion after 1 month, therefore, aspiration is not recommended if life expectancy is >1 month. Instead, it is recommended that patients with an estimated survival time of >1 month should undergo intercostal small-bore tube drainage followed by pleurodesis using intrapleural instillation of sclerosant. Cisplatin is a sclerosing agent that is widely used in the treatment of patients with lung cancer-mediated pleural effusion, exhibiting a 45-67% efficacy rate for pleurodesis (4,5). Cisplatin is characterized by good water solubility, a low local clearance rate, a low cost and direct killing of local tumor cells at different stages of the cell cycle (4). Despite the effectiveness of cisplatin in the local treatment of pleural effusion, opposing evidence indicates that the drug has a limited therapeutic effect and is unable to synergize with chemotherapeutic agents. An ideal sclerosing agent must exhibit the following characteristics: A high molecular weight and chemical

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polarity, low regional clearance, rapid systemic clearance, a steep dose-response curve and good tolerance with minimal or absent side-effects. Despite the evaluation of a wide variety of agents, no ideal sclerosing agent has been identified thus far (1). Therefore, the identification of an ideal sclerosing agent has become a major focus in studies investigating possible malignant pleural effusion treatment strategies.

p53 is an important member of the tumor suppressor family. p53 gene mutation has been identified in ~50% of human cancers, including non-small cell lung cancer (NSCLC) (6). Targeted therapy of the p53 gene and its protein product is an integral part of cancer treatment (4), as the p53 protein can inhibit tumor growth. The major functions of p53 are as follows (7-11): i) p53 blocks the cell cycle or causes the apoptosis of tumor cells; ii) introduction of the wild-type p53 gene magnifies the effect of chemotherapy and radiotherapy; iii) p53 inhibits the expression of vascular endothelial growth factor and multi-drug resistance genes, causing the apoptosis of tumor cells; iv) p53 stimulates the immune response of the human body to cancer and facilitates the local production of various types of immunocell in the tumor; and v) the p53 protein is able to kill tumor cells by cell conduction and immune system regulation, as well as the bystander effect. Previous studies have identified that targeting the p53 gene in lung cancer therapy, particularly NSCLC therapy, can achieve a satisfactory therapeutic effect (12,13).

The recombinant adenoviral human p53 gene (rAd-p53), which is widely used in the clinical treatment of lung, and head and neck cancer in mainland China (4), is a p53 adenovirus vector that has been improved by genetic engineering. rAd-p53 is a good exogenous p53 gene transportation vector, conferring high-quality pleurodesis features. A previous study proposed that the short-term efficacy of rAd-p53 plus cisplatin chemotherapy for the treatment of malignant pleural or peritoneal effusions is markedly greater than cisplatin treatment alone (14). In addition, another study demonstrated that rAd-p53 therapy for pleural or peritoneal effusion resulted in a good local effect (15). The aforementioned studies indicate that rAd-p53 therapy has good potential in malignant pleural effusion treatment, however, these previous studies used small sample sizes and chiefly focused on local effects. Thus far, only a small number of studies have been conducted investigating the feasibility, and medium- and long-term effects of rAd-53 therapy on the local treatment of lung cancer-mediated pleural effusion (4,14,15). Therefore, the present study analyzed clinical data from 210 cases of lung cancer-mediated pleural effusion treated with cisplatin (control) or rAd-p53 (experimental) for pleurodesis. The short-, medium- and long-term effects of pleurodesis in the control and experimental groups were analyzed, as well as progression-free survival (PFS) and overall survival (OS). The aim of the present study was to clarify the short-, medium- and long-term effects of rAd-p53 therapy in patients with lung cancer-mediated pleural effusion.

Patients and methods

Study subjects. Between September 2008 and December 2012, patients from Daping Hospital (Yuzhong, China) who satisfied specific inclusion criteria were recruited to the present retrospective study. The inclusion criteria were as follows: i) Histopathologically diagnosed bronchogenic carcinoma with a medium or large amount of pleural effusion; ii) an estimated survival time of >2 months; iii) an age of between 25 and 85 years; iv) an Eastern Cooperative Oncology Group performance status (PS) of 0-2 (16); v) normal liver and kidney function; vi) adoption of a chemotherapy regime of cisplatin for 2-4 cycles prior to or following pleurodesis; vii) successful pleurodesis treatment using rAd-p53 or cisplatin; viii) follow-up, including B-mode ultrasound, computed tomography (CT), positron emission tomography-CT and magnetic resonance imaging, for a period of 4-13 months after pleurodesis; and ix) relapse of pleural effusion or disease progress that was responded to in accordance with the National Comprehensive Cancer Network guidelines (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp). Furthermore, all patients provided written informed consent for participation in the present study. Using the aforementioned preliminary screening criteria, 217 patients were included. Additionally, seven cases were lost to follow-up (three rAd-p53 cases and four cisplatin cases), thus, a total of 210 cases were analyzed in the present study. The patient characteristics are summarized in Table I.

Treatment strategies. A central venous catheter was used as a drainage tube for thoracic close drainage. Prior to surgery, B-mode ultrasound was performed to determine the location of the pleural effusion.

Pleurodesis was conducted by infusing 20 ml normal saline + 100 mg lidocaine + cisplatin (or rAd-p53) into the pectoral cavity. The sclerosant dosage principles were as follows: i) 20-40 mg cisplatin or 1-$2x10^{12}$ viral particles (VP) rAd-p63. ii) Repeating the administration of the sclerosing agent was dependent on the rate of pleural effusion discharge at 24 h post-administration. For example, if the discharge rate was <100 ml/day, a central venous catheter would not be inserted; if the discharge rate was >100 ml/day, the sclerosant was infused once or more until the discharge rate was <100 ml/day. iii) The rAd-p53 dose administered in the three subgroups was $1x10^{12}$, $2x10^{12}$ and $\geq 4x10^{12}$ VP (only four and two cases were administered with $6x10^{12}$ and $8x10^{12}$ VP rAd-p53, respectively). iv) The cisplatin doses in the two subgroups were 20 and 40 mg.

In the present study, rAd-p53 (single unit dose, $1x10^{12}$ VP/ampule) was purchased from SiBiono Genetech Co., Ltd. (Shenzhen, China) and cisplatin (single unit dose, 20 mg) was obtained from Qilu Pharmaceutical Co., Ltd. (Jinan, China).

Side-effects. The two groups of patients exhibited the side-effects of fever, nausea and vomiting, chest pain, muscular soreness, leucocytopenia, and liver and kidney functional lesions.

Follow-up assessment. All patients were followed up once a month to assess for any relapse of pleural effusion, the effectiveness and safety of the therapeutic agent, and the PFS and OS times. Morphological imaging was performed during each follow-up. The response to rAd-p53 and cisplatin pleurodesis treatments was assessed using the 1979 World Health Organization criteria (www.who.int/iris/handle/10665/37200). A

Table I. Patient characteristics.

Characteristic	rAd-p53 group	Cisplatin group
Gender, n		
Male	46	75
Female	41	48
Age, years		
Range	35-80	34-81
Mean ± SD	58.1±10.4	61.8±11.1
Smoking status, n		
Ever smoker	41	61
Never smoker	46	62
PS, mean ± SD	1.092±0.583	1.049±0.625
Pathological subtype, n		
Adenocarcinoma	73	96
Squamous carcinoma	9	19
Large cell carcinoma	2	1
Small cell carcinoma	3	7
Sclerosant dose, n		
$1x10^{12}$ VP	27	
$2x10^{12}$ VP	30	
$\geq4x10^{12}$ VP	30	
20 mg		63
40 mg		60

rAd-p53, recombinant adenoviral human p53 gene; SD, standard deviation; PS, performance status; VP, viral particles.

Table II. Therapeutic effect one month after pleurodesis.

Therapeutic effect	rAd-p53 group			Cisplatin group	
	$1x10^{12}$ VP	$2x10^{12}$ VP	$4x10^{12}$ VP	20 mg	40 mg
CR, n	7	19	22	21	22
PR, n	8	4	2	13	11
NR, n	12	7	6	29	27
Side-effects, n	2	4	6	8	25

rAd-p53, recombinant adenoviral human p53; VP, viral particles; CR, complete response; PR, partial response; NR, no response.

complete response (CR) was defined as the total disappearance of pleural fluid, a partial response (PR) was defined as a decrease in the fluid volume of >50% without the requirement for drainage for >4 weeks, and no response (NR) was defined as a decrease in fluid volume of <50% or a marked increase in the volume of fluid ≤4 weeks after sclerosant administration. The overall response (OR) was calculated using the following equation: OR = CR + PR.

Statistical analysis. SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. Data were analyzed by performing χ^2 and log-rank tests. The effects of other factors on PFS were examined using the Cox proportional hazards regression model. P<0.05 was used to indicate a statistically significant difference.

Results

Therapeutic effect of pleurodesis. The therapeutic effect one month after pleurodesis is indicated in Table II. The response rate for the rAd-p53 therapy group was 71.26%, with OR in 62 cases and NR in 25 cases. By contrast, the response rate for the cisplatin therapy group was 54.47%, with OR in 67 cases and NR in 56 cases. Thus, the short-term effect of rAd-p53 therapy was significantly more favorable compared with cisplatin therapy (P<0.05). However, 12 patients in the rAd-p53 therapy

Table III. Therapeutic effect 6 and 12 months after pleurodesis.

Time after pleurodesis	rAd-p53 group, n	Cisplatin group, n
6 months		
OR	56	49
NR	31	74
12 months		
OR	28	24
NR	59	99

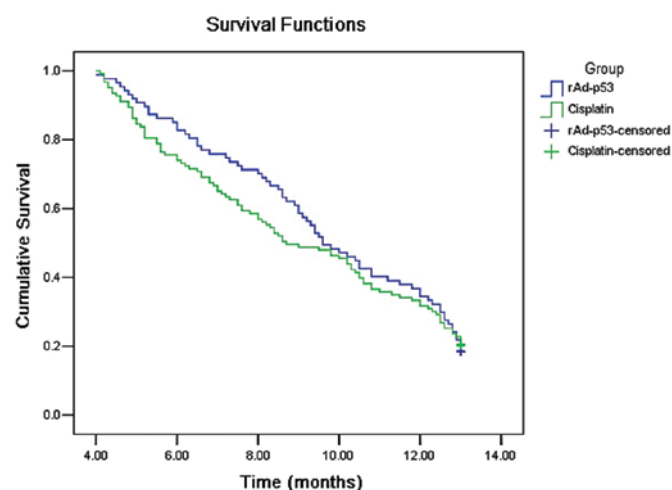rAd-p53, recombinant adenoviral human p53 gene; OR, overall response; NR, no response.

Figure 1. Overall survival curve of patients with malignant effusions.

group exhibited side-effects, accounting for 13.79% of cases; with fever identified as the main side-effect. In the cisplatin group, 33 patients suffered side-effects, accounting for 26.83% of cases. The main side-effects included fever, nausea and vomiting, and chest pain. According to subgroup analyses, the effect of 1×10^{12} VP rAd-p53 was similar to that of 40 mg cisplatin (P>0.05). Treatment with $\geq 2 \times 10^{12}$ VP was significantly more effective compared with 40 mg cisplatin (P<0.05). No significant difference was identified in the therapeutic effect of short-term pleural adhesion between treatment with 20 and 40 mg cisplatin (P>0.05).

The therapeutic effects six months after pleurodesis are indicated in Table III. The six-month OR rate for the rAd-p53 therapy group was 64.37% (56 cases), which was significantly greater than the OR of the cisplatin therapy group (39.84%; 49 cases; P<0.05).

The therapeutic effect 12 months after pleurodesis is indicated in Table III. The 12-month OR rate for the rAd-p53 therapy group was 32.18% (28 cases), which was significantly greater than in the cisplatin therapy group (19.51%; 24 cases) (P<0.05).

PFS and OS. The median PFS time was determined to be 3.2 months (95% CI, 2.785-3.615) for the rAd-p53 group and 2.7 months (95% CI, 2.096-3.304) for the cisplatin group. The difference in PFS times between the two groups was significant (P=0.008).

The median OS time was determined to be 9.6 months (95% CI, 8.483-10.717) for the rAd-p53 group and 8.7 months (95% CI, 7.147-10.253) for the cisplatin group. There was no significant difference between the two groups (P=0.630).

According to subgroup analyses, the effect of different doses of rAd-p53 on PFS were as follows: The median PFS time was determined to be 2.9 months (95% CI, 2.391-3.409) for the 1×10^{12} VP rAd-p53 subgroup, 3.3 months (95% CI, 2.629-3.971) for the 2×10^{12} VP rAd-p53 subgroup and 3.5 months (95% CI, 2.831-4.169) for the 4×10^{12} VP rAd-p53 subgroup. There was no significant difference in PFS time between the three rAd-p53 subgroups (P=0.130). In addition, subgroup analyses demonstrated the effect of different doses of cisplatin on PFS time, as follows: The median PFS was identified to be 2.6 months (95% CI, 1.822-3.378) for the 20 mg cisplatin subgroup and 2.7 months (95% CI, 1.561-3.839) for the 40 mg cisplatin subgroup. There was no significant difference between the two cisplatin subgroups (P=0.947).

Cox regression analysis. According to Cox regression analysis, age, gender and smoking status had no significant effect on PFS in the rAd-p53 group (age, P=0.444; gender, P=0.552; PS, P=0.197; and smoking status, P=0.974); however, the pathological subtypes of adenocarcinoma and squamous carcinoma did significantly affect PFS (adenocarcinoma, P=0.028; and squamous carcinoma, P=0.027). Furthermore, Cox regression analysis demonstrated that the investigated factors exerted no significant effect on PFS in the cisplatin group (age, P=0.314; gender, P=0.897; PS, P=0.252; smoking status, P=0.614; adenocarcinoma, P=0.607; and squamous carcinoma, P=0.260).

Discussion

The mean survival time of patients with malignant effusions is associated with the stage and type of the underlying malignancy (1). Patients with lung cancer-mediated malignant pleural effusion experience a relatively short survival time (2,3). Pleural effusion incurred by lung cancer metastasis is a common complication that has negative effects on lung cancer therapy and prognosis. Thus, the administration of an appropriate treatment strategy for pleural effusion is critical for improving quality of life and survival time.

Pleurodesis, a major local therapeutic strategy for malignant pleural effusion, is considered to occur through a diffuse inflammatory reaction and local activation of the coagulation system with fibrin deposition (1). In the present study, rAd-p53 was used as the sclerosing agent in the experimental group, whereas cisplatin was used as the sclerosing agent in the control group.

rAd-p53 is an easily administered sclerosing agent with a steep dose-response curve that facilitates the process of pleural adhesion. By introduction of rAd-p53 through a chest tube into the pleural space, tumor cells are infected into the pleural surface, allowing exogenous p53 genes to inhibit tumor cell growth. Thus, rAd-p53 is a good exogenous p53 gene

carrier (4). The present study demonstrates the following: i) The short-term pleurodesis effect of rAd-p53 is significantly better than that of cisplatin (P<0.05); ii) rAd-p53 pleurodesis exhibits fewer side-effects compared with cisplatin pleurodesis; iii) there is no significant difference between pleurodesis treatment with 1×10^{12} VP rAd-p53 therapy and 40 mg cisplatin therapy (P>0.05); iv) treatment with $\geq 2 \times 10^{12}$ VP rAd-p53 therapy is significantly more effective compared with 40 mg cisplatin (P<0.05); and v) different doses of rAd-p53 do not exert a significant effect on PFS. Therefore, rAd-p53 appears to be an effective agent for the local treatment of lung cancer-mediated pleural effusion. From a medical economic perspective, treatment with 2×10^{12} VP rAd-p53 is the most clinically and cost effective therapeutic dose. Considering the lack of studies that have, thus far, been conducted on the long-term effect of rAd-p53 for malignant pleural effusion, the present study performed long-term follow-up for the research subjects. Follow-up results obtained 6 and 12 months after pleurodesis demonstrated that the therapeutic effect in the rAd-p53 group was significantly greater than that in the cisplatin group (P<0.05). This results indicates that, as well as a short-term effect, rAd-p53 exhibits medium- and long-term pleurodesis effects. This may be attributed to the fact that rAd-p53 is able to facilitate and stimulate pleural adhesion, and that it is thoroughly absorbed by the pleura, thus being able to exert its anticancer function in the correct location.

The present study identified no significant difference in OS time between the rAd-p53 and cisplatin groups (P>0.05); however, the median PFS time in the rAd-p53 group was significantly higher than that in the cisplatin group (P<0.05). These results indicate that the administration of rAd-p53 may delay relapse and improve patient quality of life, although it may not prolong survival time.

According to a previous study, Inauhzin (a specific activator of the p53 pathway) may enhance sensitivity to cisplatin and reverse the multi-drug resistance of lung cancer cells (17). Additionally, there is evidence to indicate that Ad-p53 combined chemotherapy or radiotherapy has a significant therapeutic effect in advanced-stage NSCLC (18,19). The aforementioned evidence indicates that the p53 gene or protein exhibits a synergistic effect with chemotherapy or radiotherapy. In the treatment of lung cancer, rAd-p53 is typically infused via a bronchial artery or by intratumoral injection to exert this synergistic effect with chemotherapy (20,21). However, thus far, few studies have been conducted concerning the synergistic effect of chemotherapy with rAd-p53 by intrathoracic injection. The present study identified that the median PFS time in the rAd-p53 group was significantly higher than that in the cisplatin group (P<0.05), indicating that rAd-p53 by intrathoracic injection also exerts a synergistic effect with chemotherapy.

In the present study, Cox regression analysis indicated that adenocarcinoma and squamous carcinoma significantly affected PFS time in the rAd-p53 group. We hypothesize that, in the presence of marked p53 gene mutation in NSCLC, exogenous p53 introduction by rAd-p53 infusion may antagonize the mutated genes, resulting in the inhibition of tumor cell growth. Thus, in the treatment of lung cancer-mediated pleural effusion, rAd-p53 therapy may be a good treatment strategy for patients with NSCLC. However, additional large scale and multi-center clinical studies are required to verify this.

The rAd-p53 carrier is composed of human type 5 adenovirus DNA, exhibiting the lowest virulence among all the adenovirus types (22). VPs enter cells by a single infection and do not propagate or cause viral infection. Normal cells (excluding myeloid elements and epithelial mucosal cells) prevent the copying process and exhibit a markedly lower metabolism rate compared with tumor cells. As a result, the exogenous p53 gene carried by rAd-p53 is minimally expressed. Furthermore, normal cells have a complete ubiquitin degradation system for the p53 protein, therefore, any small amount of exogenous p53 protein that is expressed will be quickly degraded (23). The present study confirms the safety of rAd-p53 and verifies that pleurodesis with rAd-p53 has fewer side-effects compared with cisplatin pleurodesis.

In conclusion, rAd-p53 used in lung cancer-mediated pleural effusion treatment exerts its function as a sclerosing agent, as well as acting synergistically with chemotherapeutic agents, possibly improving patient quality of life. Subsequent studies investigating the synergistic mechanism are required. Additionally, randomized, large-scale prospective clinical studies should be conducted to verify the therapeutic effect of rAd-p53 on populations other than the Chinese population.

Acknowledgements

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